

CLAIMS

1. A method for isolating a target nucleic acid comprising
- 5 a) providing a sample containing nucleic acids,
- b) treating the sample with a lysing buffer containing a chaotropic agent to lyse cellular material in the sample, dissolve the components and denature the nucleic acids in the sample,
- 10 c) contacting the nucleic acids released from the sample with at least one capturing LNA-probe, said capturing probe being substantially complementary to the target nucleic acid.
- 15 2. A method according to claim 1, wherein the capturing LNA-probe is covalently attached to a ligand.
3. A method according to claim 1, wherein the capturing LNA-probe is covalently attached to a solid surface.
- 20 4. A method according to claim 2, wherein the ligand covalently attached to the LNA-probe is coupled to an anti-ligand, said anti-ligand being covalently attached to a solid surface.
- 25 5. A method according to claims 3 or 4, wherein after the contact of the nucleic acids released from the sample with the LNA attached to the solid surface, the solid surface is separated from excess material.
6. A method according to claim 5, wherein the solid surface is washed with buffer to re-
- 30 move excess material.
7. A method according to any of the preceding claims, wherein the capturing probe is complementary to the target nucleic acid.

8. A method according to any of the preceding claims, wherein the capturing probe is between 4 and 50 nucleotides long.
9. A method according to any of the preceding claims, wherein the capturing probe is between 8 and 30 nucleotides long.
10. A method according to any of the preceding claims, wherein the capturing probe is between 8 and 20 nucleotides long.
- 10 11. A method according to any of the preceding claims, wherein the capturing probe is between 8 and 15 nucleotides long.
12. A method according to any of the preceding claims, wherein more than one capturing LNA-probe is used and the capturing probes consist of different LNA-oligomers directed against different target nucleic acids or against different regions of the same nucleic acid.
- 15 13. A method according to claim 12, wherein the different capturing probes are spotted in an array format on the solid surface.
- 20 14. A method according to claim 13, wherein the array has at least 10 capturing probes.
15. A method according to claim 13, wherein the array has at least 100 capturing probes.
16. A method according to claim 13, wherein the array has at least 1,000 capturing probes.
- 25 17. A method according to claim 13, wherein the array has at least 10,000 capturing probes.
- 30 18. A method according to any of the preceding claims, wherein the nucleic acids originate from cells, a tissue sample or a tissue extract.
19. A method according to claim 18, wherein the cells are of archae, prokaryotic, eukaryotic origin.
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20. A method according to claim 18, wherein the sample is derived from blood, serum, plasma, reticulocytes, lymphocytes, urine, bone marrow tissue, cerebrospinal fluid or any product prepared from blood or lymph, muscle biopsy, liver biopsy, kidney biopsy, bladder biopsy, bone biopsy, cartilage biopsy, skin biopsy, pancreas biopsy, a biopsy of the intestinal tract, thymus biopsy, mammae biopsy, uterus biopsy, a testicular biopsy, eye biopsy or a brain biopsy, homogenized in lysis buffer.

21. A method according to any of the preceding claims, wherein the capturing probe(s) consist(s) of LNA-oligomer(s) directed against one or more target nucleic acid(s) which is/are specific for a specific species of organisms.

22. A method according to any of the preceding claims, wherein the capturing probe(s) consist(s) of LNA-oligomer(s) directed against one or more target nucleic acid(s) which is/are specific for a specific species, sub-species or strain of organisms.

23. A method according to any of the preceding claims, wherein the capturing probe(s) consist(s) of LNA-oligomer(s) directed against one or more target nucleic acid(s) which is/are specific for a specific species of micro-organisms.

24. A method according to any of the preceding claims, wherein the capturing probe(s) consist(s) of LNA-oligomer(s) directed against one or more target nucleic acid(s) which is/are specific for a specific species, sub-species or strain of micro-organisms.

25. A method according to any of the preceding claims, wherein the capturing probe(s) consist(s) of LNA-oligomer(s) directed against one or more target nucleic acid(s) which is/are specific for an infectious agent.

26. A method according to any of the preceding claims, wherein the capturing probe(s) consist(s) of LNA-oligomer(s) directed against one or more target nucleic acid(s) which is/are specific for a species, sub-species or strains of an infectious agent.

27. A method according to any of the preceding claims, wherein the capturing probe(s) consist(s) of LNA-oligomer(s) directed against one or more target nucleic acid(s) which is/are specific for genes coding for proteins involved in an inheritable disease.

28. A method according to any of the preceding claims, wherein the capturing probe(s) consist(s) of LNA-oligomer(s) directed against one or more target nucleic acid(s) which is/are specific for genes related to a life style disease.
- 5 29. A method according to any of the preceding claims, wherein the capturing probe(s) consist(s) of LNA-oligomer(s) directed against one or more target nucleic acid(s) which is/are specific for genes related to cancer.
30. A method according to claim 28, wherein the life style diseases are selected from the
10 group consisting of atherosclerosis and diabetes.
31. A method according to any of the preceding claims, wherein the solid surface is selected from the group consisting of glass, carbohydrate polymers and metals.
- 15 32. A method according to any of the preceding claims, wherein the solid surface is the wall of a well in a microtiter-plate.
33. A method according to any of the preceding claims, wherein the solid surface has the form of a bead.
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34. A method according to any of the preceding claims, wherein the solid surface has the form of a flat plate.
35. A method according to any of the preceding claims, wherein the isolation is performed
25 in one step.
36. A method according to any of claims 2 and 3, wherein the ligand is biotin.
37. A method according to any of the preceding claims, wherein the target nucleic acids
30 hybridised to the capturing probe are detected by a detection probe.
38. A method according to claim 37, wherein the detection probe is labelled with a label selected from the group consisting of fluorophores, radioactive isotopes, enzymes, ligands and haptenic and antigenic compounds.
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39. A method according to claim 38, wherein the fluorophore is selected from the group consisting of fluorescein, rhodamin and Texas Red.

40. A method according to claim 38, wherein the radioactive isotope is selected from the group consisting of ^{32}P , ^{33}P , ^{35}S , ^3H , ^{125}I and ^{14}C .

41. A method according to claim 38, wherein the enzyme is selected from the group consisting of horseradish peroxidase, alkaline phosphatase, calf intestine alkaline phosphatase, glucose oxidase and beta-galactosidase.

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42. A method according to claim 38, wherein the ligand is selected from the group consisting of biotin, thyroxine and cortisol.

43. A method according to any of claims 37 to 42, wherein the detection probe hybridises to a different region of the immobilised target nucleic acid than the capturing probe.

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44. A method according to any of claims 37 to 42, wherein the detecting probe contains at least one LNA-monomer.

45. A method for amplifying a target nucleic acid the nucleotide sequence of which is complementary to the capturing probe, the method comprising the steps of

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a) providing a sample containing nucleic acids,

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b) treating the sample with a lysing buffer containing a chaotropic agent to lyse cellular material in the sample, dissolve the components and denature the nucleic acids in the sample,

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c) contacting the nucleic acids released from the sample with at least one capturing LNA-probe covalently attached to a solid surface, said capturing probe being substantially complementary to the target nucleic acid,

d) separating the solid surface from excess material,

e) combining the captured nucleic acids with an appropriate amount of nucleoside triphosphates and an agent for polymerisation of the nucleoside triphosphates,

5 f) extending any oligonucleotides which hybridise to the captured nucleic acids to form extension products, wherein the capturing LNA-probes are used as templates,

g) detecting the extension product formed.

10 46. A method for amplifying a target nucleic acid the nucleotide sequence of which is complementary to the capturing probe, the method comprising the steps of

a) providing a sample containing nucleic acids,

15 b) treating the sample with a lysing buffer containing a chaotropic agent to lyse cellular material in the sample, dissolve the components and denature the nucleic acids in the sample,

20 c) contacting the nucleic acids released from the sample with at least one capturing LNA-probe covalently attached to a solid surface, said capturing probe being substantially complementary to the target nucleic acid,

d) separating the solid surface from excess material,

25 e) combining the captured nucleic acids with an appropriate amount of nucleoside triphosphates and an agent for polymerisation of the nucleoside triphosphates,

30 f) extending any oligonucleotides which hybridise to the captured nucleic acids to form extension products, wherein the capturing LNA-probes are used as templates,

35 g) hybridising, in the presence of an appropriate amount of nucleoside triphosphates and an agent for polymerisation of the nucleoside triphosphates, the single stranded nucleic acids from step c) with at least one downstream primer to synthesise further extension products,

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h) repeating steps g) through h) a sufficient number of times to result in a detectable amount of extension products,

5 i) detecting the extension products formed.

47. A method for amplifying a target nucleic acid the nucleotide sequence of which is complementary to the capturing probe, the method comprising the steps of

10 a) providing a sample containing nucleic acids,

b) treating the sample with a lysing buffer containing a chaotropic agent to lyse cellular material in the sample, dissolve the components and denature the nucleic acids in the sample,

15 c) contacting the nucleic acids released from the sample with at least one capturing LNA-probe covalently attached to a solid surface, said capturing probe being substantially complementary to the target nucleic acid,

20 d) separating the solid surface from excess material,

e) combining the captured nucleic acids with an appropriate amount of nucleoside triphosphates, an agent for polymerisation of the nucleoside triphosphates and at least one downstream primer,

25 f) extending any oligonucleotides which hybridise to the captured nucleic acids to form extension products, wherein said nucleic acids are used as templates,

30 g) detecting the extension product formed.

48. A method for amplifying a target nucleic acid the nucleotide sequence of which is complementary to the capturing probe, the method comprising the steps of

35 a) providing a sample containing nucleic acids,

b) treating the sample with a lysing buffer containing a chaotropic agent to lyse cellular material in the sample, dissolve the components and denature the nucleic acids in the sample,

5 c) contacting the nucleic acids released from the sample with at least one capturing LNA-probe covalently attached to a solid surface, said capturing probe being substantially complementary to the target nucleic acid,

10 d) separating the solid surface from excess material,

e) combining the captured nucleic acids with an appropriate amount of nucleoside triphosphates, an agent for polymerisation of the nucleoside triphosphates and at least one downstream primer,

15 f) extending any oligonucleotides which hybridise to the captured nucleic acids to form extension products, wherein said nucleic acids are used as templates,

20 g) hybridising, in the presence of an appropriate amount of nucleoside triphosphates and an agent for polymerisation of the nucleoside triphosphates, the single stranded nucleic acids from step c) with at least one downstream primer to synthesise further extension products,

h) repeating steps g) through h) a sufficient number of times to result in a detectable amount of extension products,

25 i) detecting the extension products formed.

49. A kit for isolating a target nucleic acid comprising

30 a) a lysing buffer containing a chaotropic agent to lyse cellular material in the sample,

b) at least one capturing LNA-probe, said capturing probe being substantially complementary to the target nucleic acid.

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